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Autoradiographic Localization of [^{125}I]-Ricin in Lungs and Trachea of Mice Following an Aerosol Inhalation Exposure

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13. ABSTRACT (*Maximum 200 words*)
Studies were conducted to examine the light microscopic distribution of [^{125}I]-ricin within the lungs and corresponding lung pathology in mice following single, nose-only, aerosol inhalation exposures. Groups of 4-8 mice were euthanized 15 minutes and 1, 4, 6, 8, 12, and 24 hours post-exposure. At 15 minutes post-exposure, abundant radiolabel was present within alveolar spaces. At all later times, however, [^{125}I]-ricin was observed primarily within the lumina of bronchioles and larger passageways. Cellular targets for ricin within the lungs could not be identified using the techniques employed; it is therefore recommended that ultrastructural autoradiography be used to determine cellular ricin binding targets. The primary pathological abnormality detected was multifocal epithelial necrosis of the secondary bronchioles.

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Introduction

The inhalation of aerosolized ricin is believed to represent a potentially significant hazard to military personnel (Cope et al., 1946; Quinchon and Levy, 1962; Korosuo, 1971). The primary biochemical lesion produced by ricin is inactivation of protein synthesis (Olsnes et al., 1974). However, the precise sites of toxin action remain unknown. Recent studies conducted in our laboratories (Doebler et al., 1995) have suggested that the lungs may represent the only pathophysiologically important site of [^{125}I]-ricin accumulation following an aerosol exposure. This hypothesis is consistent with previous results indicating that histopathological abnormalities are essentially restricted to the lungs following an aerosol exposure to ricin (Griffiths et al., 1993).

The current investigation was conducted to determine the microscopic distribution of [^{125}I]-ricin in mice following aerosol inhalation exposures and to relate ricin binding to pathological findings.

Materials and Methods

Ricin (Vector Laboratories, Burlingame, CA) was iodinated with carrier-free [^{125}I] (Amersham, Arlington Heights, IL) using the lactoperoxidase method (Marchalonis, 1969). Extensive studies were conducted previously to validate the use of [^{125}I]-ricin as a probe for toxin localization (Doebler et al., 1995). The toxicity of [^{125}I]-ricin was confirmed both in vitro (using inhibition of protein synthesis and inhibition of cellular proliferation as endpoints) and in vivo (using a two-dosage regimen with survival as endpoint). In addition, the degradation of [^{125}I]-ricin in mouse tissues was found to be substantial (69-74% of radiolabel in liver and spleen was precipitated in 10% trichloroacetic acid at 60 min post-injection), but not so severe as to preclude the use of [^{125}I]-ricin as a probe for toxin localization (Doebler et al., 1995).

Twenty-eight male CD-1 mice (weighing 18-22 grams) were obtained from Harlan Sprague-Dawley Inc. (Detrick, MD). Upon arrival, mice were quarantined and observed for evidence of disease for 5 days before use. Mice were housed in polycarbonate shoebox cages in groups of 10 or less per cage. They were provided commercial certified mouse ration and tap water ad libitum. Animal holding rooms were maintained at 21 ± 2 °C on a 12-hour light/dark cycle. All mice received [^{125}I]-ricin (specific activity 1.72 X 10^9 cpm/mg) by nose-only aerosol inhalation. Groups of 4 mice were euthanatized (as described below) at 15 minutes, 1, 4, 8, 12 and 24 hours after [^{125}I]-ricin exposure; an additional group of 4 mice was euthanatized at 1 hour post-exposure (total n for 1 hour = 8). Aerosols of ricin (13 mg/minute/meter^3) were generated by a Collison nebulizer operated at 26 pounds per square inch (psi) of air pressure, giving 7.5 liters/minute with mass median aerodynamic diameter (MMAD) of 1.06 μm (geometric standard deviation =1.203) as measured by an Aerodynamic Particle Sizer, APS3000 (TSI Inc., St. Paul, MN). Total flow rate through the non-rebreathing system was adjusted to 12 liters/minute with a secondary air flow of 4.5 liters/minute. Aerosol sampling was conducted continuously for the duration of the 10-minute exposure using an all glass impinger (AGI) with a sonic velocity of 6.0 liters/minute. The ricin exposure level was sublethal (Estep, unpublished data) and did not produce overt signs of toxicity in any of the exposed animals.

Mice were euthanatized by pentobarbital overdose (6.5 mg/mouse, ip), and blood was collected (until heart stoppage) by snipping the tail. Lungs were removed in toto, frozen immediately in a dry ice-ethanol slurry and stored on dry ice. Entire lungs were mounted and sectioned (at 10 μm) on a cryostatic microtome. Approximately every 10th or 15th section was saved onto a glass slide; this frequency was determined by the presence of large intrapulmonary passageways in the immediate vicinity of the section.

Preliminary studies were conducted to determine whether sufficient resolution could be obtained using X-ray film for the autoradiography of [^{125}I]-ricin administered by aerosol inhalation in the lungs of mice. Dupont (Wilmington, DE) Chronex and Ultra-Vision G films were placed in direct apposition to cryostatic sections of lung obtained from exposed mice. However, following development of the autoradiograms, it was obvious that the resolution provided by either film was not sufficient to localize the [^{125}I] label into various compartments of the lung. Thus, an autoradiographic emulsion (Kodak NTB-3 Nuclear Track Emulsion, Rochester, NY) was used in subsequent studies for the localization of [^{125}I]-ricin. Tissues on slides were covered with NTB-3 emulsion, placed vertically to allow even drying, and stored in the dark for 28 days. Routine developing procedures (using Kodak D-19 developer) were employed at the end of the exposure period. Sections were then counterstained with hematoxylin-eosin for microscopic examination.

Results and Discussion

Despite the markedly enhanced resolution provided by the emulsion relative to the films, specific cellular compartments accumulating [^{125}I]-ricin within the lung could not be identified with any degree of certainty. However, the label could be readily identified in specific intrapulmonary air passageways. This distribution is briefly described below.

The distribution of [^{125}I]-ricin within the lungs differed markedly among groups of mice sacrificed at 15 minutes and those groups sacrificed at all later times. In the 15-minute post-exposure group, abundant radiolabeled material was detected within the alveolar spaces and in the lumina of bronchioles, bronchi and the trachea. At later time periods (1-24 hours post-exposure), [^{125}I]-ricin was essentially restricted to the lumina of bronchioles and larger respiratory passageways. The disappearance of radiolabel from the alveolar spaces after the early post-exposure interval may signify the actual absorption of [^{125}I]-ricin into the alveoli.

No pathologic abnormalities could be detected in the lungs of mice sacrificed prior to 24 hours post-exposure. However, bronchiolar lesions were detected at 24 hours post-exposure. These bronchiolar lesions consisted of sites of minimal multifocal epithelial necrosis (observed primarily in secondary bronchioles) and were characterized by condensation of cytoplasm, pyknosis and disorganization of the epithelium. Some sloughing of the epithelium was also present. Occasional neutrophils were observed transmigrating the mucosa and submucosa.

The major objective of these studies was to determine the cellular distribution of [^{125}I]-ricin binding and corresponding histopathological abnormalities within the lungs of mice following nose-only aerosol inhalation exposures. However, the procedures employed were not sensitive enough to localize toxin or toxin-induced damage to specific cell types within the lungs. We believe that such information could be readily obtained using electron microscopic

autoradiography combined with routine morphological analyses. With regard to toxin localization, we have found that light microscopic autoradiography, performed with either film or emulsions, did not provide sufficient resolution to identify specific cell types. Since previous attempts to localize ricin using immunocytochemical techniques have also not been successful (D.R. Franz, personal communication), it appears that ultrastructural autoradiographic procedures, such as those described by Salpeter and Bachmann (1972), are required to determine ricin localization within specific cell types. We were also unable to identify any specific respiratory epithelial components within the lung that may have been selectively damaged by aerosol-administered ricin. The relatively thick (10 μm) frozen sections made detailed evaluation of respiratory epithelial subtypes difficult. It is likely that routine morphological processing procedures (i.e., formalin-fixed tissues and 5-7 μm paraffin sections) can be used to obtain additional insight as to specific lung cells damaged by aerosolized ricin.

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